

Calculation of energy levels and electron distribution in reduced and oxidized forms of diphosphopyridine nucleotide and of flavine

By BARBRO GRABE

With 7 figures in the text

SUMMARY

Energy levels and electron densities are calculated in different oxidation states of diphosphopyridine nucleotide and flavine-adenine dinucleotide, both of which take part in the oxidative processes in the living cell. Only those parts of the molecules where oxidation and reduction take place are considered in the calculation. The π -electrons and some lone pairs in these parts are considered explicitly. Molecular orbitals (MO) for these " π -electrons" are formed by linear combination of atomic orbitals (LCAO). The antisymmetrized products (AP) of these orbitals are used. Some of the integrals in the electronic wave equation are determined semi-empirically according to a treatment by Pariser and Parr [1] and by Pople [2]. The self-consistent field method (SCF) is used for solving the equations. In the discussion it is shown that the results seem to give considerable support to the hypothesis of the present writer [3] concerning the oxidative phosphorylation at the diphosphopyridine nucleotide and flavine molecules in the living cell.

I. Introduction

In the present paper energy levels and electron distributions have been calculated in different oxidation states of diphosphopyridine nucleotide and flavine-adenine dinucleotide which take part in oxidative processes in the living cell. Most substances in the living cell are not oxidized directly by oxygen. Instead there is a chain of carriers which transfer electrons or hydrogen atoms from the substance to oxygen. These carriers alternate between the oxidized and the reduced states, thus being reduced by the nearest neighbour on the substrate side and oxidized by the nearest neighbour on the oxygen side. Diphosphopyridine nucleotide and flavine-adenine dinucleotide are known to be neighbours in such a chain, diphosphopyridine nucleotide being oxidized by the flavine. They are very large molecules. However, only the

Abbreviations used: DPN⁺ and DPNH diphosphopyridine nucleotide in oxidized and reduced form; DPNH⁺ the free radical obtained if an electron is removed from DPNH; FAD and FADH₂ flavine-adenine dinucleotide in oxidized and reduced form and FAD[•] the free radical obtained if an electron is transferred to FAD.

part of the molecule which changes oxidation state has been included in the present considerations, i.e. the pyridine ring of the diphosphopyridine nucleotide and the isoalloxazine ring of the flavine. The structure of the actual parts of the compounds is given in Fig. 1, the rest of the molecules being denoted R. The abbreviations DPN⁺, DPNH, FAD and FADH₂ given in the figure are used in the following. Since it has been suggested in [3] that an electron is transferred from DPNH to FAD at an intermediate state in the oxidation in the cell, the free radicals DPNH⁺ and FAD⁻ have been treated also, although it is most probable that there is a charge transfer complex between DPNH and FAD built in the cell and no DPNH⁺ and FAD⁻ free radicals.

Pullman and Pullman [4] have previously calculated the electron distribution and energy levels in different oxidation states of the diphosphopyridine nucleotide and of flavine mononucleotide, the latter having the same flavine part as FAD. They have, however, used other methods and parameter values than the present author. Their calculations include more electrons than the present, but on the other hand they have used a more approximate method, viz. the Hückel approximation. It will therefore be of interest to compare the results of the different calculations. (See Section IV.)

In Section II a description of the calculation is given. Thus II a) includes an outline of the method used and states which atoms are included in the calculation and which electrons are considered explicitly. In II b) the wave equation for the π -electrons is given and II c) includes parameter values chosen according to experimental data and a description of the evaluation of some integrals. In II d) the method for the solution of the wave equation is given and in II e) and f) equalities for the calculations of electron densities, bond orders and total energies are given. The results are given in Section III and discussed in Section IV, where the hypothesis in [3] is discussed with respect to the present results.

II. Calculation

a) *An outline of the method used*

The method used for the calculation is a semi-empirical LCAO-MO-AP-SCF method. This implies that molecular orbitals (MO) have been built by linear combination of atomic orbitals (LCAO) and that the ordinary method with antisymmetrized products (AP) of MO:s has been used according to Roothaan [5a]. For the free radicals with unpaired electrons the extended calculation by Roothaan [5b] has been followed. Instead of calculating all the integrals a semi-empirical method given by Pariser and Parr [1] and by Pople [2] has been used. However, no configuration interaction has been taken into consideration as Pariser and Parr have done. For solution of the equations the self-consistent field (SCF) treatment has been used. More details are given in the following.

As is mentioned in Section I, the calculation includes only parts of the large molecules, i.e. only the atoms numbered in Fig. 1 are considered.

The usual π -approximation has been used, i.e. the interaction of all the π -electrons and lone pairs at some of the N and O atoms have been considered explicitly, the effect of the σ -electrons being included in the term of the Hamiltonian denoted H^{core} . Thus for DPN⁺ 6 electrons have been considered explicitly (i.e. the π -electrons), for DPNH 8 electrons (i.e. the π -electrons plus the lone pair at the N atom plus 2 electrons in the C=H₂ bond, the H₂ group being treated as a unity [6]), for FAD 10

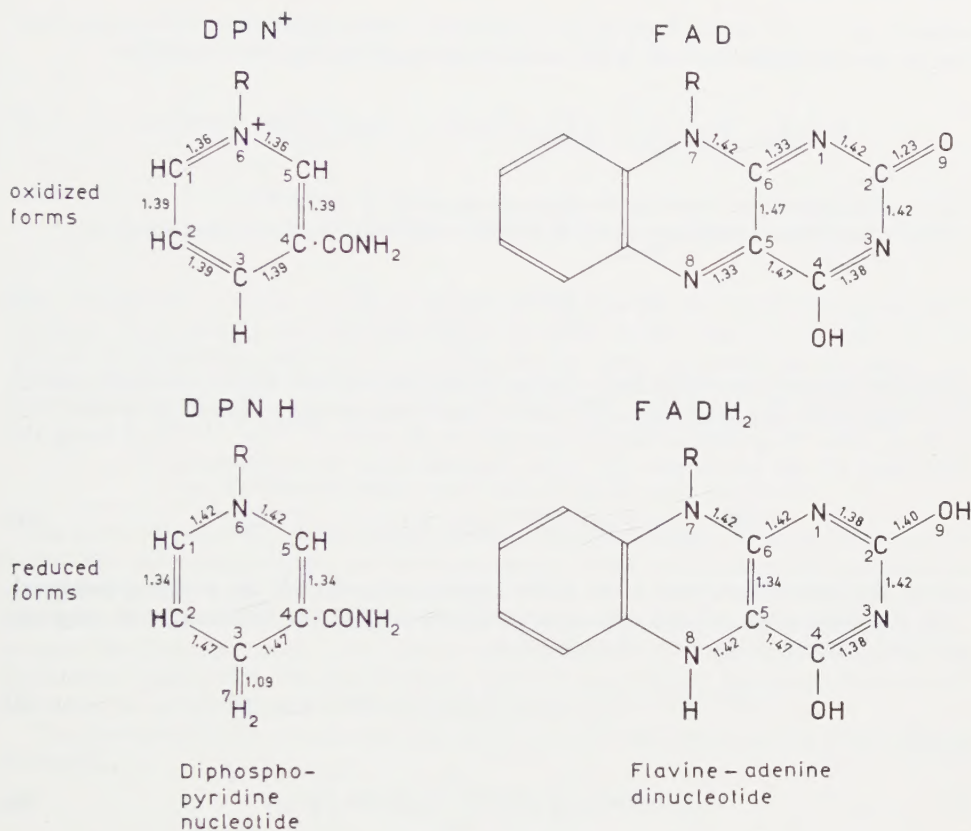


Fig. 1. The numbers give the interatomic distances, in Å, used in the calculations. They are estimated from distances in similar bonds, given in the literature.

electrons (i.e. 8 π -electrons plus the lone pair at the N atom denoted (7) in Fig. 1) and for FADH₂ 12 electrons (i.e. 6 π -electrons plus the lone pairs at N(7), N(8) and O(9) in Fig. 1). The free radical DPNH⁺ has been calculated as DPNH, where one electron from the occupied orbital of highest energy has been removed, and FAD⁻, as FAD in which one electron has been transferred to the empty π -orbital of lowest energy from a reducing substance. In these cases, therefore, 7 electrons and 11 electrons respectively have been considered explicitly. All the electrons considered explicitly are denoted π -electrons.

b) The wave equation for the π -electrons

To obtain MO:s for the π -electrons atomic orbitals $\chi_p(\mu)$, where the index p indicates the atom to which the orbital belongs and μ indicates the electron considered, have been combined to orthonormal molecular space orbitals, $\varphi_i(\mu)$

$$\varphi_i(\mu) = c_{i1}\chi_1(\mu) + c_{i2}\chi_2(\mu) + \dots + c_{is}\chi_s(\mu), \quad (1)$$

where 1, 2, ... s includes all the atoms considered in the calculation. These $\varphi_i(\mu)$ have finally been antisymmetrized in the usual manner, giving the wave function

$$\psi(1, 2, \dots, n) = \frac{(-1)^P}{\sqrt{n!}} \sum P \varphi_i(1) \varphi_j(2), \dots, \varphi_v(n) \times \text{spin functions}, \quad (2)$$

where n indicates the number of electrons considered explicitly.

The total Hamiltonian operator H for the electrons has been expressed as

$$H = \sum_{\mu} H^{\text{core}}(\mu) + \frac{1}{2} \sum_{\mu} \sum_{v \neq \mu} \frac{e^2}{r_{\mu v}}, \quad (3)$$

where the summation over μ and v has to be carried out over all the electrons considered explicitly. The expression $H^{\text{core}}(\mu)$ is the kinetic energy operator of the electron μ , $T(\mu)$, plus the potential energy operator of the core, $U^{\text{core}}(\mu)$, $U^{\text{core}}(\mu)$ being the field from all the nuclei plus the inner electrons plus the σ -electrons.

$$H^{\text{core}}(\mu) = T(\mu) + U^{\text{core}}(\mu) = T(\mu) + \sum_s U_s^{\text{core}}(\mu), \quad (4)$$

where the summation over s has to be carried out over all the atoms considered.

In the cases where all occupied space orbitals contain two electrons with opposite spin the problem is to solve the equation

$$[H^{\text{core}}(\mu) + 2 \sum_j J_j(\mu) - \sum_j K_j(\mu)] \varphi_i(\mu) = \varepsilon_i \varphi_i(\mu), \quad (5)$$

where

$$J_j(\mu) \varphi(\mu) = \left[\int \varphi_j^*(v) \frac{e^2}{r_{\mu v}} \varphi_j(v) dv \right] \varphi(\mu) \quad (6)$$

and

$$K_j(\mu) \varphi(\mu) = \left[\int \varphi_j^*(v) \frac{e^2}{r_{\mu v}} \varphi(v) dv \right] \varphi_j(\mu). \quad (7)$$

The summation over j in (5) has to be carried out over all the occupied space orbitals. $-\varepsilon_i$ is the ionization energy for removal of an electron in φ_i .

For the calculation of the electron distribution in DPNH^+ and FAD^- , where one electron is unpaired, the method of Roothaan [5b] has been used. In applying his method to the present case it is found that the problem is to solve the following equations

$$\left[H^{\text{core}}(\mu) + 2 \sum_j J_j(\mu) - \sum_j K_j(\mu) + J_e(\mu) - \frac{1}{2} K_e(\mu) + M_e(\mu) \right] \varphi_k(\mu) = \varepsilon_k \varphi_k(\mu) \quad (8a)$$

for the paired electrons and

$$[H^{\text{core}}(\mu) + 2 \sum_j J_j(\mu) - \sum_j K_j(\mu) + 2 \sum_j M_j(\mu)] \varphi_e(\mu) = \varepsilon_e \varphi_e(\mu) \quad (8b)$$

for the unpaired electron. J_j and K_j are defined as in (6) and (7) and

$$\begin{aligned}
 M_j(\mu) \varphi(\mu) &= \frac{1}{2} \{ [\int \varphi_j^* K_e \varphi dv] \varphi_j(\mu) + [\int \varphi_j^* \varphi dv] K_e(\mu) \varphi_j(\mu) \} = \\
 &= \frac{1}{2} \left\{ \left[\int \varphi_j^*(\lambda) \varphi_e^*(v) \frac{e^2}{r_{\lambda v}} \varphi(v) \varphi_e(\lambda) dv_\lambda dv_v \right] \varphi_j(\mu) + \right. \\
 &\quad \left. + \int \varphi_j^*(\lambda) \varphi(\lambda) dv_\lambda \int \varphi_e^*(v) \frac{e^2}{r_{\mu v}} \varphi_j(v) dv_v \varphi_e(\mu) \right\}. \quad (9)
 \end{aligned}$$

The summation over j in (8) has to be carried out over all the doubly occupied space orbitals. The index e denotes the singly occupied orbital and J_e , K_e and M_e are defined in accordance with (6), (7) and (9). In the cases calculated in this paper $-\varepsilon$ is approximately equal to the ionization energy, although, according to Roothaan [5b], this is not true in the general case, when there are unpaired electrons.

c) Parameter values and evaluation of some integrals

To solve (5) and (8) it is necessary to know the interatomic distances in the molecules. The interatomic distances between nearest neighbours used in the calculations are given in Fig. 1. It should be pointed out that there seems not to be any experimentally determined distances given in the literature, as far as the author is aware, so that the distances given in the figure are estimated by comparison with distances in similar bonds. For the free radicals DPNH⁺ and FAD⁻ the same interatomic distances as in DPNH and FAD have been used.

The first step in the calculation has been to determine the values of the following integrals.

$$\alpha_p = \int \chi_p^*(\mu) H^{\text{core}}(\mu) \chi_p(\mu) dv_\mu \quad (10)$$

$$\beta_{pq} = \int \chi_p^*(\mu) H^{\text{core}}(\mu) \chi_q(\mu) dv_\mu \quad (11)$$

and

$$(pr|qs) = \int \chi_p^*(\mu) \chi_q^*(v) \frac{e^2}{r_{\mu v}} \chi_r(\mu) \chi_s(v) dv_\mu dv_v. \quad (12)$$

Following Pariser and Parr [1], (10) and (11) are assumed to be parameters, to be determined semi-empirically. Furthermore, the calculation has been simplified by assuming tentatively that $\beta_{pq} = 0$, when p and q are non-neighbours, and that $(pr|qs) = 0$, when $p \neq r$ and $q \neq s$, i.e. only integrals of the type $(pp|qq)$ are taken into consideration. The last assumption implies a formal neglect of differential overlap, i.e. $\chi_p^* \chi_r = 0$ for $p \neq r$. Furthermore, in α_p the terms $\int \chi_p^*(\mu) U_q^{\text{core}}(\mu) \chi_p(\mu) dv_\mu$ (see (10) and (4)), when $p \neq q$, have been calculated as

$$\int \chi_p^*(\mu) U_q^{\text{core}}(\mu) \chi_p(\mu) dv_\mu = \int \chi_p^*(\mu) U_q^{\text{neutr}}(\mu) \chi_p(\mu) dv_\mu - N_q (pp|qq),$$

where $N_q = 1$ or 2, according to the number of electrons contributed to the π -system by the atom q . The first integral in the right-hand member will always be small. Following Pople [2] we have neglected this integral in the present computations. Thus

$$\alpha_p = -I_p - \sum_{q \neq p} (pp|qq), \quad (13)$$

where $-I_p$ is the ionization potential of the valence state of the free atom. The summation over q has been carried out over all the electrons considered explicitly.

Following Pariser and Parr [1] and Anno [7] the ionization potentials and the β -values have been taken from empirical data. The values used are given in Table 1. The I_C and I_N have been taken from Anno [7] and I_{N+} chosen = 26.2 eV. I_O has been taken from Skinner and Pritchard [8] and I_{H_2} from [9]. The β -values are dependent on the distance between the atoms. For β_{CC} the values have been taken from Pariser and Parr [1], who have given β_{CC} -values for two distances and a formula for determining values for other distances. For β_{CN} the value -2.74 eV for the distance 1.35 Å has been used, in accordance with Anno's suggestions [7]. To get β_{CN} -values for other distances, it has been assumed that the curve giving the dependence of β_{CN} on the interatomic distance is parallel to the corresponding curve for β_{CC} . β_{CO} for the distance 1.23 Å has been taken from Sidman [10]. For the distance 1.40 Å it has been supposed that $\beta_{CO}(r = 1.40 \text{ Å}) = \beta_{CN}(r = 1.40 \text{ Å})$. The value of β_{CH_2} has been obtained by calculating the integrals in

$$\beta_{qp} = H_{pq}^{\text{core}} - (S_{pq}/2) (H_{pp}^{\text{core}} + H_{qq}^{\text{core}}). \quad (14)$$

See footnote in [1a]. For β_{CN+} the value -3.6 eV has been chosen.

The expressions $(pp|qq)$ have been determined according to formulas given by Pariser and Parr [1]. For interatomic distances $r \geq 2.80$ Å they have given the following formula obtained from the uniformly charged sphere model:

$$(pp|qq) = 7.1975/r \{ [1 + (1/2r)^2 (R_p - R_q)^2]^{-1/2} + [1 + (1/2r)^2 (R_p + R_q)^2]^{-1/2} \} eV, \quad (15)$$

R_p and R_q being diameters of nonconducting spheres. They are to be determined as

$$R_s = 4.597 / Z_s (\text{in Å}), \quad (16)$$

Z_s being Slater's effective nuclear charge. To get a corresponding value on R_{H_2} the present author has determined the diameter R_{H_2} by comparing the ball values and the exact values of integrals at $r = 0$ (compare Anno [7]). All the R_s values used in the present paper are given in Table 2.

Table 1. Values of ionization energies, I , and of resonance integrals, β , (11), used for calculations.

	I_C		I_N		I_{N+}		I_{H_2}		I_O	
eV	11.54		14.00		26.2		15.6		17.21	
a.u.	0.4242		0.5146		0.962		0.573		0.6326	

Interatomic distances, Å	β_{CC}			β_{CN}				β_{CO}		β_{CH_2}
	1.34	1.39	1.47	1.33	1.36	1.38	1.42	1.23	1.40	1.09
eV	-3.16	-2.39	-1.51	-3.05	-2.60	-2.37	-1.95	-3.0	-1.95	-2.64
a.u.	-0.1162	-0.0879	-0.0555	-0.1121	-0.0956	-0.0871	-0.0717	-0.110	-0.0717	-0.096

Table 2. Diameters used in (15).

	C	N	N ⁺	O	H ₂
<i>R</i>	1.41	1.18	1.08	1.01	1.8

For the determination of $(pp|qq)$ for $r \leq 2.80$ Å Pariser and Parr [1] have given the following formula for extrapolation:

$$(pp|qq) = 1/2[(pp|pp) + (qq|qq)] - ar - br^2, \quad (17)$$

where $(pp|pp)$ and $(qq|qq)$ are to be obtained from experimentally determined ionization energies, I , of valence states of the free atom and electron affinities, A , as

$$(ss|ss) = I_s - A_s. \quad (18)$$

The parameters a and b are to be determined from values of $(pp|qq)$ for large r values, e.g. for $r = 2.80$ Å and $r = 3.70$ Å, calculated according to (15).

The parameter values used in (17) in the present paper are given in Table 3 for different atoms p and q .

 Table 3. Formulas for determination of $(pp|qq)$ in eV, according to (17), when the interatomic distance $r \leq 2.80$ Å.

(CC CC)	$= 11.08 - 2.9226 r + 0.26203 r^2$	(According to Anno [7])
(CC NN)	$= (11.08 + 13.46)/2 - 3.6807 r + 0.3747 r^2$	
(CC N ⁺ N ⁺)	$= (11.08 + 17.21)/2 - 4.841 r + 0.5518 r^2$	(17.21 according to Anno [7])
(CC H ₂ H ₂)	$= (11.08 + 15.62)/2 - 4.4708 r + 0.5054 r^2$	
(CC OO)	$= 12.757 - 3.966 r + 0.4169 r^2$	(According to Sidman [10])
(NN NN)	$= 13.46 - 4.3907 r + 0.4813 r^2$	
(NN OO)	$= (13.46 + 14.67)/2 - 4.741 r + 0.5323 r^2$	(14.67 according to Sidman [10])

d) The solution of the electronic wave equation

To start the SCF calculation a zero-order approximation of the c coefficients of (1) are needed for the insertion in J , K and M in (5) and (8). To get such values a calculation in the Hückel approximation has been carried out, i.e. all the electrons are included in H^{core} , so that all the $e^2/r_{\mu\nu}$ terms in (3) disappear. In each cycle of the SCF calculation the solving of the system of equations corresponding to (5) or (8) to get the ε and c values has been made on the Swedish electronic computer BESK. The calculation has been continued until the difference between the ε -values in the last two cycles are at most 0.002 a.u., ensuring at least two and in most cases three decimals in the electron densities calculated from the c values.

e) Determination of electron densities, charges on the atoms and bond orders

The final c values in (1) have been used to calculate the electron densities at the different atoms according to Coulson [11]. His definition of "charge" is identical

with Mulliken's definition [12] of gross atomic population $N(r)$ on the atom r when the overlap integrals are neglected:

$$N(r) = \sum_j N_j c_{jr}^2. \quad (19)$$

The summation should be carried out over the occupied space orbitals and N_j should be 2 for doubly occupied orbitals and 1 for singly occupied orbitals.

The charge $Q(r)$ on the atom r is defined as

$$Q(r) = N_O(r) - N(r), \quad (20)$$

where $N_O(r)$ is the number of electrons in the analogous orbitals of the neutral atom.

The bond orders p_{rs} have been calculated according to Coulson [11] and [13]:

$$p_{rs} = \sum_j N_j c_{jr} c_{js}. \quad (21)$$

The value of N_j and the summation over j are as in (19). When the summation over j is over orbitals occupied by π -electrons only, p_{rs} is called mobile bond order.

f) Energy values

As mentioned above, the orbital energies obtained by solution of (5) and (8) are equal to the ionization energy for removal of an electron in the corresponding orbital.

The total π -electron energy for the case when all occupied orbitals contain two electrons is

$$E_\pi = \sum_i H_i^{\text{core}} + \sum_i \varepsilon_i. \quad (22)$$

The summation should be carried out over all occupied space orbitals.

For the special cases FAD^- and DPNH^+ , where an electron is unpaired, the total π -electron energy is

$$E_\pi = \sum_j H_j^{\text{core}} + 1/2 H_e^{\text{core}} + \sum_j \varepsilon_j + 1/2 \varepsilon_e. \quad (23)$$

The summation over j should be carried out over all doubly occupied space orbitals and e indicates the singly occupied orbital.

We are interested in differences in total energy between $\text{DPNH} + \text{FAD}$ (I), $\text{DPNH}^+ + \text{FAD}^-$ (II) and $\text{DPNH}^+ + \text{FADH}_2$ (III). The total energy, E , consists of the π -electron energy and the core energy, and in II, also the electrostatic energy, E_{st} , which is dependent on the relative orientation of the charged radicals. Assuming that the same interatomic distances occur in I and II, the core energy is approximately the same in I and II. The core energy of III is, however, different, partly due to changed interatomic distances and partly due to the formation of new σ -bonds. It therefore seems possible to calculate only the energy difference between I and II with the present method. When calculating the energy difference

$$E(\text{I}) - E(\text{II}) = E_\pi(\text{I}) - E_\pi(\text{II}) - E_{st} \quad (24)$$

the electrostatic energy, E_{st} , is calculated on the assumption that the radicals are situated in two parallel planes, the O(9) of FAD^- situated above N of the pyridine

ring of DPNH^+ as in Fig. 7, and that the charges are distributed according to Figs 2 and 3. The distance between the planes is assumed to be 3.3 Å. (Hausser and Murrell [14] assume that the corresponding distance between two similar free radicals is between 3 and 3.5 Å.)

III. Results

As mentioned above, a calculation in the Hückel approximation has first been carried out for each substance to get c values for the first cycle of the SCF calculation. The results of these Hückel calculations are given together with the final results from the semi-empirical method according to Pariser and Parr [1] to get a comparison between the methods. This latter method is denoted the SCF approximation. The Hückel approximation for the free radicals DPNH^+ and FAD^- is obtained

Table 4. Orbital energies ε in a.u. obtained with the semiempirical method, denoted the SCF approximation, according to (5) and (8), and in the Hückel approximation.

DPN^+		DPNH		FAD		FADH_2		DPNH^+	FAD^-
SCF	Hückel	SCF	Hückel	SCF	Hückel	SCF	Hückel	(free radical) SCF	(free radical) SCF
Occupied orbitals									
						-0.661	-0.670		-0.422
				-0.613	-0.695	-0.580	-0.626		-0.357
		-0.603	-0.635	-0.557	-0.628	-0.518	-0.578	-0.846	-0.322
0.913	-1.030	-0.515	-0.602	-0.541	-0.591	-0.499	-0.556	-0.745	-0.276
0.700	-0.790	-0.487	-0.540	-0.483	-0.572	-0.429	-0.526	-0.708	-0.240
0.694	-0.780	-0.414	-0.489	-0.449	-0.520	-0.390	-0.461	-0.667*	-0.101*
Empty orbitals									
0.265	-0.655	-0.006	-0.370	-0.088	-0.422	-0.021	-0.374		
0.246	-0.585	+0.021	-0.308	-0.016	-0.349	+0.019	-0.337		

* Only an unpaired electron in this orbital.

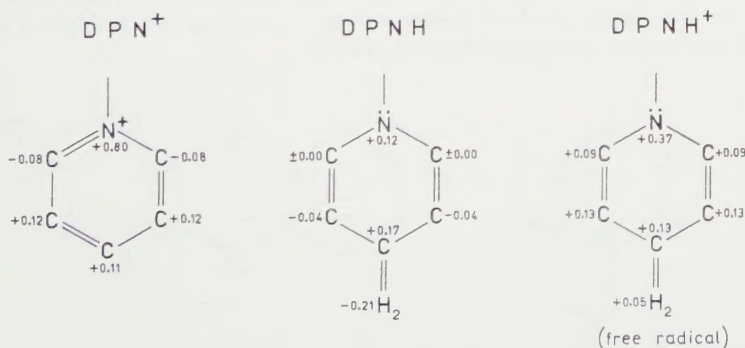


Fig. 2. The numbers give the charges on the atoms according to (20), with the electrons considered explicitly. The values are obtained with the SCF method.

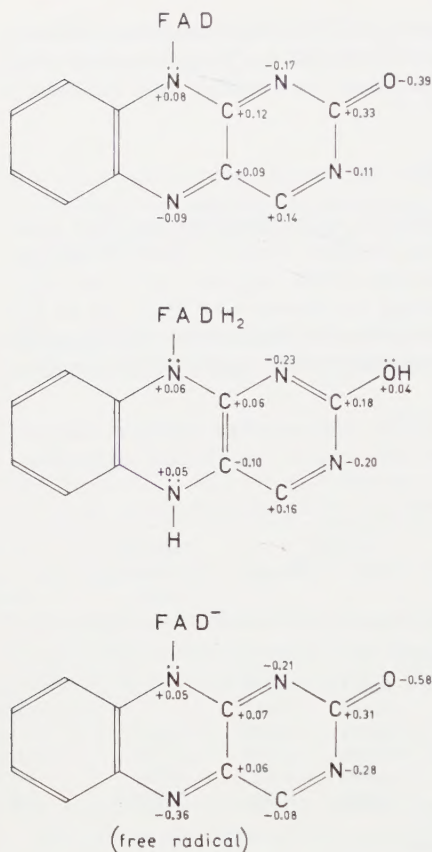


Fig. 3. The numbers give the charges on the atoms according to (20), with the electrons considered explicitly. The values are obtained with the SCF method.

directly by removal of an electron from the highest occupied level of DPNH and by transferring an electron to the lowest empty level of FAD.

The orbital energies ϵ are given in Table 4 for the levels occupied in the ground state and for the empty levels of lowest energy. $-\epsilon_i$ gives the ionization energy for removal of an electron occupying the i :th orbital.

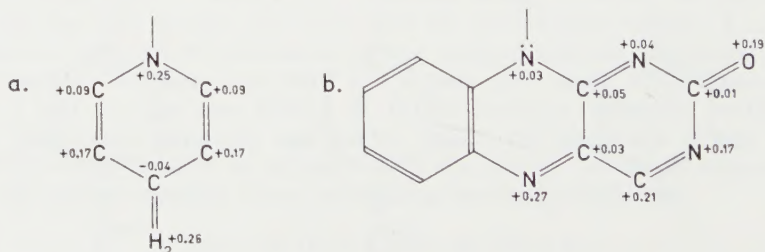


Fig. 4. a) Differences in gross atomic population between DPNH and DPNH⁺, calculated with the SCF method. b) Differences in gross atomic population between FAD⁻ and FAD, calculated with the SCF method.

Table 5. The gross atomic population $N(r)$ according to (19) with the electrons considered explicitly.

$N(r)$ on atom	DPN ⁺		DPNH		DPNH ⁺ (free radical)	
	SCF	Hückel	SCF	Hückel	SCF	Hückel
C(1) and C(5) respectively	1.08	1.14	1.00	0.90	0.91	0.89
C(2) and C(4) respectively	0.88	0.73	1.04	1.10	0.87	0.90
C(3)	0.89	0.49	0.83	0.62	0.87	0.54
N(6)	1.20	1.78	1.88	1.66	1.63	1.27
H ₂ (7)	—	—	1.21	1.72	0.95	1.62

	FAD		FADH ₂		FAD ⁻ (free radical)	
	SCF	Hückel	SCF	Hückel	SCF	Hückel
N(1)	1.17	1.46	1.23	1.51	1.21	1.46
C(2)	0.67	0.50	0.82	0.68	0.69	0.64
N(3)	1.11	1.41	1.20	1.45	1.28	1.47
C(4)	0.86	0.50	0.84	0.69	1.08	0.82
C(5)	0.91	0.68	1.10	1.06	0.94	0.81
C(6)	0.88	0.66	0.94	0.93	0.93	0.74
N(7)	1.92	1.74	1.94	1.87	1.95	1.79
N(8)	1.09	1.29	1.95	1.90	1.36	1.47
O(9)	1.39	1.76	1.96	1.92	1.58	1.80

The distribution of the electrons considered explicitly, i.e. the gross atomic populations of these electrons, is given in Table 5. The charges Q on the atoms are given in Figs 2 and 3. The differences in the atomic populations between DPNH and DPNH⁺ and between FAD⁻ and FAD are given in Fig. 4. The mobile bond orders according to (21) are given in Figs 5 and 6.

The difference in π -electron energy between DPNH + FAD (I) and DPNH⁺ + FAD⁻ (II) is -0.24 a. u. This value is obtained with the semi-empirical method accord-

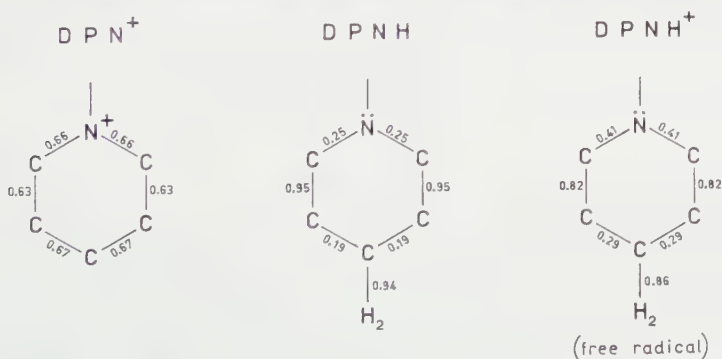


Fig. 5. The numbers along the bonds give the mobile bond orders according to (21), with the SCF method.

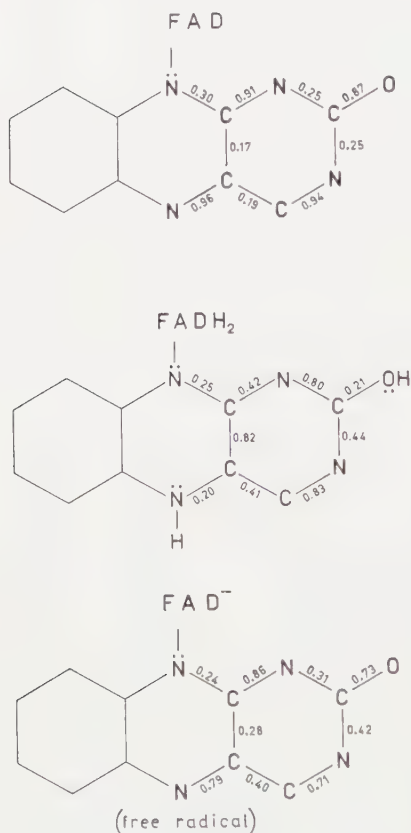


Fig. 6. The numbers along the bonds give the mobile bond orders according to (21), with the SCF method.

ing to Pariser and Parr [1], here denoted the SCF approximation. The electrostatic energy for $\text{DPNH}^+ + \text{FAD}^-$ is 0.12 a.u., if the distance between the planes is 3.3 Å (for the configuration in space see Fig. 7). The total energy difference between (I) and (II) is therefore -0.24 a.u. = -6.5 eV for large distances between the charged radicals and -0.12 a.u. = -3.3 eV for the distance 3.3 Å, on the assumption that the core energy is unchanged and that no charge transfer complex is formed. The formation of such a complex is discussed in IV.

IV. Discussion

From Table 4 it is seen that the ionization energies obtained with the Hückel approximation are larger than the values obtained with the semi-empirical method according to Pariser and Parr, denoted the SCF approximation. The differences between the values are especially large for the empty orbitals. For the occupied orbitals the agreement between the methods is comparatively good, the differences varying between 0.03 and 0.12 a.u. In the following discussion we shall only consider the values from the method denoted the SCF approximation.

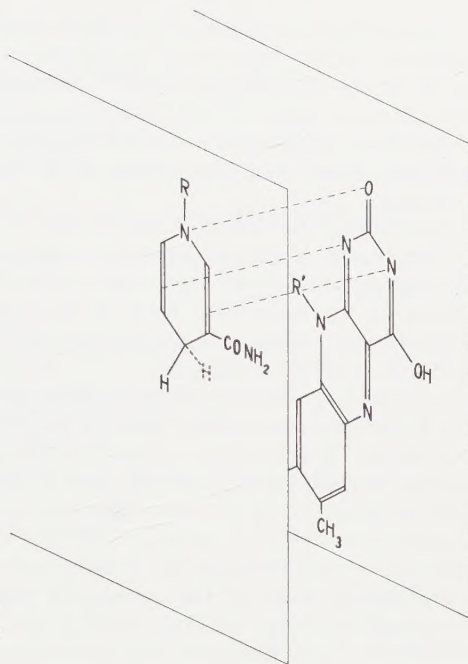


Fig. 7. Assumed orientation in two parallel planes of the free radicals DPNH^+ and FAD^- , when the electrostatic energy is calculated.

It is seen that the ionization energies of an electron from DPN^+ and from FAD are very large (0.694 and 0.449 a.u. respectively), for DPNH and FADH_2 the corresponding values being lower, i.e. 0.414 and 0.390 a.u. respectively. The orbital energies of the lowest empty levels of DPN^+ and FAD are -0.265 and -0.088 a.u. respectively, the corresponding values for DPNH and FADH_2 being only -0.006 and -0.021 a.u. respectively. These results correspond well with the facts that DPNH and FADH_2 are easily oxidized, i.e. they are good electron donors, and that DPN^+ and FAD are easily reduced, i.e. they are good electron acceptors.

Pullman and Pullman [4] have only given K values for the orbital energies, where $\varepsilon = \alpha + K\beta$. According to a private communication they estimate $-\beta$ to be approximately 20 Kcal/mole and at most 40 Kcal/mole. If a value within this interval is used for $-\beta$ and our value -0.4242 a.u. for α , the ε -values obtained with Pullman's K -values for DPNH , FAD and FADH_2 agree well with our SCF values for the occupied orbitals of highest energy and with our Hückel values for the empty orbitals of lowest energy.¹ This is reasonable, since Pullman and Pullman have used a Hückel approximation. For DPN^+ however the ε -values obtained from the K values of [4] differ considerably from our values, the value for the highest occupied level being -0.46 a.u. and for the lowest empty level -0.41 a.u. if $-\beta = 20$ Kcal/mole is used. The poor agreement may be explained by the difficulties in choosing parameter values for N^+ in DPN^+ .

Those atomic charges which are of special interest are the charges on the N atom of the pyridine ring of the diphosphopyridine nucleotide, and of N(8) and O(9) of the flavine. (Index numbers in parenthesis refer to Fig. 1.)

¹ The discrepancies at most 0.03 a.u.

Considering the diphosphopyridine nucleotide, it is seen that the charge on N increases by 0.25 units when an electron is removed from DPNH, so that the charge on N in the free radical DPNH^+ becomes $+0.37$. In DPN^+ it is further increased by 0.43 to a value of 0.80. Pullman and Pullman [4] have calculated the electron distribution in the free radical DPN, i.e. when an electron is transferred to DPN^+ , and they have found the difference in charge on N between DPN^+ and DPN to be 0.18 units.

Considering the flavine, it is seen that the negative charge on N(8) in FAD increases by 0.27 units, when an electron is transferred to the lowest empty orbital, so that the charge on N(8) in FAD^- is -0.36 . The negative charge on O(9) in FAD increases at the same time by 0.19, so that the charge on O(9) in FAD^- is -0.58 . Pullman and Pullman [4] have found the increase in negative charge on N(8) to be 0.28 and on O(9) to be 0.02.

In [3] an hypothesis is made for the manner in which a high-energy phosphate bond, due to the oxidation taking place in the DPN-FAD-part of the respiratory chain, can be formed. The hypothesis is based on the assumptions that a charge transfer complex can be formed between DPNH^+ and FAD^- , and that phosphate can be bound to the O(9) of FAD^- , if at the same time the electrons of O^- in the phosphate ion are displaced towards the N atom of DPNH^+ (N is supposed to be positively charged) so that the σ -bond between the P atom and the latter O atom is weakened. It is then a significant result of the calculation that the positive charge on N in DPNH and the negative charge on O(9) in FAD are increased by transfer of an electron from DPNH to FAD. However, it is seen from the results that the energy of $\text{DPNH}^+ + \text{FAD}^-$ (II) is higher than that of $\text{DPNH} + \text{FAD}$ (I). If the first step of oxidation of DPNH by FAD is an electron transfer in the supposed manner, the energy of (II) must be lowered e.g. by formation of a charge transfer complex. In [15] Mulliken has made an approximate calculation on a pair of molecules, viz. an electron donor, benzene (denoted B), and an electron acceptor, iodine (denoted A). In this case the energy difference between $\text{A}^- + \text{B}^+$ and $\text{A} + \text{B}$ is 8 eV, when the components are a large distance from each other, and between 4 and 5 eV when the distance between the components is 3.4 Å. Mulliken maintains that his calculations give considerable support to the theory that the formation of a charge transfer complex causes the observed absorption at λ 2900. Certainly this calculation refers to other molecules than the present ones, but since the energy differences are of the same order of magnitude as the energy differences found in the present calculations between I and II, it seems to be very probable that in the present case overlap between the states I and II may cause a lowering of the energy by a complex formation. The present calculation thus gives considerable support to the hypothesis in [3].

ACKNOWLEDGEMENTS

The author wishes to thank Dr. Inga Fischer-Hjalmars for her interest and encouragement and for her helpful guidance. The author is also indebted to Mr. Lars Norén for his painstaking performance of the routine calculations on the BESK computer. The research, upon which this paper is based, was performed under a grant from Statens Naturvetenskapliga Forskningsråd.

Institute for Theoretical Physics, University of Stockholm, December 1959.

REFERENCES

1. R. PARISER and R. G. PARR, a) J. Chem. Phys. 21 (1953), 466, b) *ibid.* 767.
2. J. A. POPLER, Trans. Far. Soc. 49 (1953), 1375.
3. B. GRABE, Biochim. Biophys. Acta 30 (1958), 560.
4. B. PULLMAN and A. PULLMAN, Proc. National Acad. Sciences 45 (1959), 136.
5. C. C. J. ROTHMAN, a) Rev. Mod. Phys. 23 (1951), 69, b) Reported at the Boulder Conference, June 1959.
6. R. S. MULLIKEN, C. A. RIEKE and W. G. BROWN, J. Amer. Chem. Soc. 63 (1941), 41.
7. T. ANNO, J. Chem. Phys. 29 (1958), 1161.
8. H. A. SKINNER and H. O. PRITCHARD, Trans. Far. Soc. 49 (1953), 1254.
9. G. HERZBERG, Molecular Spectra and Molecular Structure. I. Spectra of Diatomic Molecules. New York 1950.
10. J. W. SIDMAN, J. Chem. Phys. 27 (1957), 429.
11. C. A. COULSON, Valence, Oxford At the Clarendon Press, 1953.
12. R. S. MULLIKEN, J. Chem. Phys. 23 (1955), 1833.
13. C. A. COULSON, Proc. Roy. Soc. 169 A (1939), 413.
14. K. H. HAUSSER and J. N. MURRELL, J. Chem. Phys. 27 (1957), 500.
15. R. S. MULLIKEN, J. Amer. Chem. Soc. 74 (1952), 811.

Tryckt den 27 februari 1960

Uppsala 1960. Almqvist & Wiksells Boktryckeri AB

